

SHORT COMMUNICATION

Exploring the compatibility between *Kluyveromyces lactis* and probiotic *Lactobacillus* spp. and their In-vitro antimicrobial potential

Venus Bansal^a(✉), Pranav Kumar Singh^a, Santosh Kumar Mishra^b, Nitika Goel^a, Gajanan P Deshmukh^c, S Sivakumar^c and Manvesh Kumar Sihag^d

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Abstract: This study investigated symbiotic interactions between dairy yeast and probiotic *Lactobacillus* spp., where stable coexistence occurs through the shared utilization of common metabolites and environmental conditions. The primary objective was to evaluate the compatibility between the potential probiotic yeast *Kluyveromyces lactis* (*K. lactis*) and probiotic *Lactobacilli* strains, namely *Lacticaseibacillus paracasei* subsp. *paracasei*, *Lactobacillus acidophilus*, *Lacticaseibacillus rhamnosus*, *Lactiplantibacillus plantarum* subsp. *plantarum*, and *Lactobacillus helveticus*. Additionally, the antibacterial activity was explored. The spot assay on MRS agar confirmed equal growth intensity and the absence of incompatibility between *K. lactis* and probiotic *Lactobacilli* spp. Their cultures mutually promoted each other's growth. Most probiotic *Lactobacillus* strains exhibited significant antibacterial activity against tested pathogens, while *K. lactis* showed no antibacterial activity. Among six strains of *Lactobacillus*, *L. rhamnosus* displayed the maximum zone of inhibition (cm) with 3.063 ± 0.071 , 2.754 ± 0.133 , 2.818 ± 0.125 against *B. cereus*, *S. aureus* and *E. coli*, respectively. The minimum activity was observed in *L. acidophilus* and *L. bulgaricus* with no zone of inhibition against *S. aureus* by both bacteria. The findings of this study on the symbiotic interactions and compatibility of dairy yeast and probiotic *Lactobacillus* strains, as well as their enhanced growth and significant antibacterial activity, provide important insights for the development of innovative probiotic products with potential health benefits.

Keywords: Compatibility, Antimicrobial, Yeast, *Lactobacillus*, Probiotic

Introduction

Probiotic-based products represent the future of the food market. It is evidenced from the fact that the worldwide probiotics market, which reached a valuation of USD 77.12 billion in 2022, is projected to experience a compound annual growth rate of 14.0% from 2023 to 2030 (Grand View Research, 2023). The *Bifidobacteria* and *Lactobacillus* spp. are the most commonly employed for the development of probiotic based food products. Nonetheless, due to numerous research' findings about the possible health advantages of yeasts, the market for probiotic formulations based on yeast has grown (Czerucka et al., 2007). Although, *Sacchromyces* is the only proven yeast as a probiotic, the recent studies report evidence that yeast spp. like *Kluyveromyces marxianus* and *Pichia kudriavzevii* may have probiotic properties (Staniszewski & Kordowska-Wiater, 2021). This is primarily attributed to their enhanced resistance to diverse environmental challenges, reduced likelihood of acquiring and transmitting antibiotic resistance, and distinct immune signaling to the host in comparison to probiotics based on lactic acid bacteria (Oliveira et al., 2017).

The widely recognized advantages of probiotics are frequently ascribed to the interaction between the probiotic that is delivered and the microbiota of the gastrointestinal tract (GI). The diversity and makeup of gut microbes have a significant impact on host health because they can affect the synthesis of enzymes, the generation of metabolites like vitamins and short-chain fatty acids,

^aDepartment of Dairy Technology, College of Dairy and Food Science Technology, Guru Angad Dev Veterinary & Animal Sciences University, Ludhiana-141004, Punjab, India.

^bDepartment of Dairy Microbiology, College of Dairy and Food Science Technology, Guru Angad Dev Veterinary & Animal Sciences University, Ludhiana-141004, Punjab, India.

^cDepartment of Dairy Engineering, College of Dairy and Food Science Technology, Guru Angad Dev Veterinary & Animal Sciences University, Ludhiana-141004, Punjab, India.

^dDepartment of Dairy Chemistry, College of Dairy and Food Science Technology, Guru Angad Dev Veterinary & Animal Sciences University, Ludhiana-141004, Punjab, India.

^eDivision of Research Systems Management, ICAR-National Academy of Agricultural Research Systems Management, Hyderabad, India

Venus Bansal (✉)

Department of Dairy Technology, College of Dairy Science & Technology, Guru Angad Dev Veterinary & Animal Sciences University, Ludhiana-141004, Punjab. Mobile: +91-94784-76400, Email: venusbansal@gadvasu.in

the control of cell-to-cell interactions, the immune system and neuroendocrine responses, and the digestion and absorption of nutrients (Washburn et al., 2022). However, just as no single food can fully meet the needs of the human body, likewise, no individual probiotic is sufficient to deliver comprehensive health benefits to consumers. According to Washburn et al. (2022), microbial gastrointestinal diversity is not substantially impacted by the administration of a single species probiotic in healthy people. Consequently, the emerging trend in the market is the utilization of a combination of probiotic-based products. Primarily, the blending of probiotic *Lactobacillus* varieties is employed to offer a diverse range of health advantages. Nevertheless, the combination of yeast and *Lactobacillus* spp. has been employed historically in the production of cheese and other food products. However, formulations of probiotics containing both yeast and *Lactobacillus* spp. have been relatively underexplored.

Kluyveromyces, particularly *Kluyveromyces lactis*, has emerged as a pivotal yeast genus in both research and industrial biotechnology. Frequently found in milk and cheese, these yeasts are naturally ingested along with these foods (Andrade et al., 2017; Ceugniz et al., 2017; Fadda et al., 2017). This genus exhibits resistance to passage through the gastrointestinal tract (GI) and demonstrates potential for adhesion to the intestinal epithelium. Additionally, it possesses functional properties, including the production of short-chain fatty acids, immune modulation, inhibition of pathogens, and pro-apoptotic activity in cancerous epithelial cells (Kumura et al., 2004; Maccaferri et al., 2012; Ceugniz et al., 2017; Saber et al., 2017). Most recently, Gut et al. (2019) has studied the probiotic properties of *K lactis* and stated that it has potential probiotic characteristics comparable with established probiotic yeast i.e., *Saccharomyces boulardii*.

Therefore, this study attempted to evaluate the mutual compatibility between probiotic *Lactobacillus* and *Kluyveromyces lactis*, with the overarching objective of establishing a harmonious interaction between yeast and probiotic *Lactobacillus* strains. The investigation also encompasses an assessment of their respective antibacterial properties, particularly in relation to potential pathogenic microorganisms.

The probiotic yeast culture *Kluyveromyces lactis* (MTCC 458) was purchased from the Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh. The *Lactobacillus* strains viz., *Lacticaseibacillus paracasei* subsp. *paracasei*, *Lactobacillus acidophilus*, and *Lacticaseibacillus rhamnosus* of probiotic cultures were procured from Chr. Hansen A/S, 10-12 Boege Alle, DK-2970 Hoersholm, Denmark. The other probiotic strains i.e. *Lactiplantibacillus plantarum* subsp. *plantarum*, *Lactobacillus bulgaricus* and *Lactobacillus helveticus* were obtained from NCDC, NDRI, Karnal. *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), and *Bacillus cereus* (*B. cereus*) cultures

were procured from Department of Dairy Microbiology, College of Dairy Science and Technology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana.

Compatibility between different strains of probiotic cultures was checked by spot assay on MRS Agar. The probiotic cultures were taken in active log phase of their growth. An aliquot of 3 µL broth from both cultures was spotted adjacent to each other on agar to check the compatibility between two cultures. Plates were incubated at 37 °C for overnight and after incubation intensity of growth in spots was observed to check any inhibition or spontaneity into the growth zone of bacteria.

Antagonistic activity of probiotic strains against *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), and *Bacillus cereus* (*B. cereus*) was assessed by the cut well diffusion assay as per the protocol of Zhang et al., (2011) with partial modifications. Briefly, cell free supernatant (CFS) from each of the probiotic strains was collected by centrifugation of overnight grown culture at 8,000 g for 10 min. An aliquot of 100 µl of actively growing individual strains *E. coli* and *S. aureus* in BHI broth (log phase culture) containing 10⁷ cfu/ml of pathogen were seeded in 7 mL of molten soft BHI agar, (0.5 %) mixed gently and poured in BHI agar plates. 1.25 cm diameter wells were punched in agar plates using a sterile borer and an aliquot of 200 µl of the supernatants from each probiotic strain was added separately in their respective wells. Un-inoculated MRS broth adjusted at pH 6.8 served as the negative control. Supernatants were allowed to diffuse into agar for few minutes by storing the plates in a refrigerator (7°C) and the plates were then transferred in an incubator set at 37°C in an inverted position till growth free inhibition zones appeared around the wells. The zone diameters (in cm) reflecting the antibacterial activity was measured with the help of an imageJ software. One way analysis of variance (ANOVA) was performed using minitab 18 statistical software to find significant differences in the zone diameters. Means with p-value < 0.05 were considered statistically different.

Figure 1 shows the compatibility between *K lactis* and probiotic *Lactobacilli* strains. After being incubated overnight at 37 °C, both cultures in the spot exhibited nearly equal growth intensity. These findings are supported by the growth pattern of the cultures which clearly demonstrates the absence of any incompatibility between *K lactis* and the probiotic *Lactobacilli* spp. Moreover, it was observed that *K lactis* and the probiotic *Lactobacilli* spp. mutually promoted each other's growth. This is evident from the thicker colony observed at the intersection of their cultures compared to the individual cultures (Fig. 1). The results are in agreement with the previous studies reported by Shimizu et al. (2006) and Menezes et al. (2018). Shimizu et al. (2006) studied the symbiotic relationship between *Lactococcus lactis* and *K lactis* and reported harmonious relationship between each other. Furthermore, Menezes et al. (2018) specified that yeasts contribute to LAB growth, and vice versa, since they provide

Table 1: Antibacterial activity of probiotic *Lactobacillus* strains against *B cereus*, *S aureus* and *E coli* using cut well assay

Strain	Zones of inhibition (cm) including well diameter (1cm)		
	<i>B cereus</i>	<i>S aureus</i>	<i>E coli</i>
LA	1.987 ± 0.302 ^d	ND	2.444 ± 0.198 ^{bc}
LB	2.197 ± 0.217 ^{cd}	ND	2.397 ± 0.138 ^{bc}
LC	2.723 ± 0.29 ^{abc}	2.043 ± 0.077 ^b	2.511 ± 0.141 ^{abc}
LH	2.353 ± 0.19 ^{bcd}	2.009 ± 0.199 ^b	2.31 ± 0.115 ^c
LG	3.063 ± 0.071 ^a	2.754 ± 0.133 ^a	2.818 ± 0.125 ^a
LP	2.829 ± 0.415 ^{ab}	2.572 ± 0.199 ^a	2.74 ± 0.285 ^{ab}

Zone of inhibition including well diameter (1 cm); ND- Not detected; Means with small letter superscripts within a column shows significant (p<0.05) differences in the zone diameters

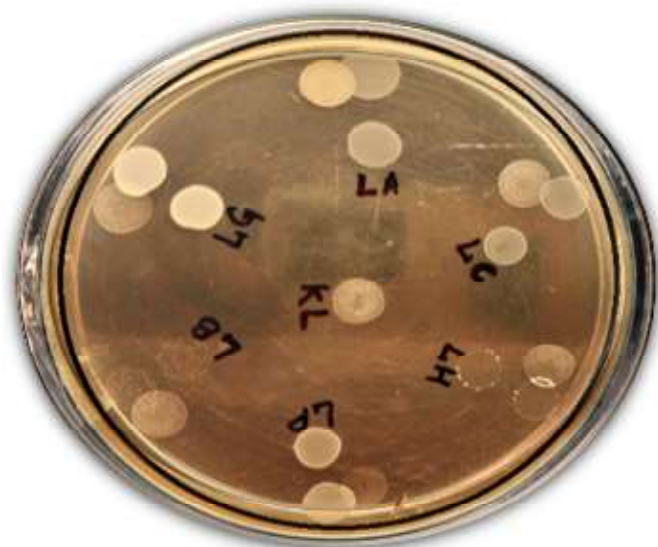


Fig. 1 Compatibility between *K lactis* and probiotic *Lactobacilli* spp.; LA- *L acidophilus*; LC- *L casei*; LH- *L helveticus*; LP- *L plantarum*; LB- *L bulgaricus*; LG- *L rhamnosus*

some nutrients, such as amino acids and vitamins to LAB. The compatibility between dairy yeast *K Lactis* and probiotic *Lactobacilli* needs to be further explored in different type of food matrices to confirm their symbiotic attributes that can be harnessed in the developments of novel functional foods.

Table 1 shows the zone of inhibition of probiotic *Lactobacillus* strains against *B. cereus*, *S. aureus* and *E. coli* using cut well assay. It could be observed from the table 1 that among six different strains of *Lactobacillus*, *L rhamnosus* displayed the maximum zone of inhibition (cm) with 3.063 ± 0.071, 2.754 ± 0.133, 2.818 ± 0.125 against *B. cereus*, *S. aureus* and *E. coli*, respectively. The minimum activity was observed in *L acidophilus* and *L bulgaricus* with no zone of inhibition against *S aureus* by both bacteria. The results of the study indicated that most of the probiotic *Lactobacilli* strains displayed significant antibacterial

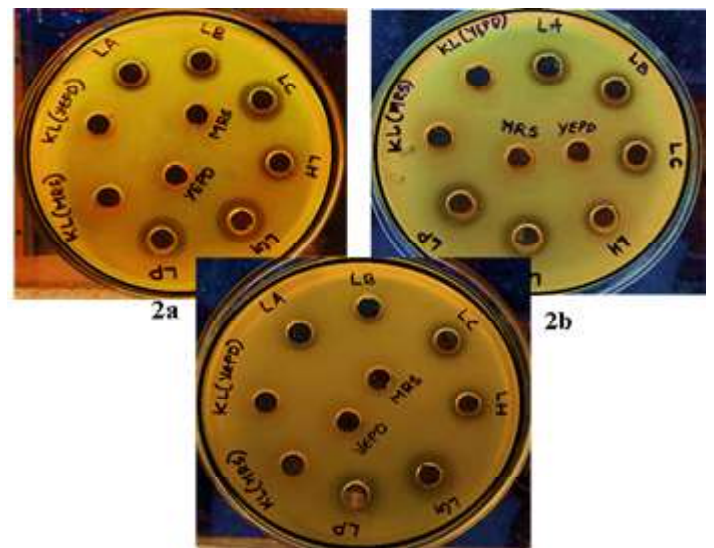


Fig. 2 Agar plates showing inhibitory activity of probiotic *Lactobacillus* strains against tested pathogens; 2a) *B cereus*; 2b) *E coli*; 2c) *S aureus*

activity against the tested pathogens. However, the inhibition spectra varied among the different strains, as evident from the diverse sizes of the zones of inhibition shown in Figure 2. The results are in accordance with the previous studies where authors have reported antibacterial effect of different *Lactobacillus* spp. against various pathogens (Fayol-Messaoudi et al., 2005; Zhang et al., 2011). The antibacterial effect of *Lactobacillus* spp. could be due to excessive acid production and other microbial component including bacteriocins which become active in acidic pH conditions.

However, it is noteworthy that the cut well assay method employed to evaluate the antimicrobial properties of *K lactis* yielded no growth inhibition against *E. coli*, *S. aureus*, and *B. cereus*, as illustrated in Figure 2. These findings align with the results reported by Gut et al. (2019), where no antibacterial activity

against *E. coli* ATCC 43895 and *Enterobacter aerogenes* VUN 00025 was observed. Conversely, Gut et al. (2022) reported antimicrobial potential of *K lactis* against *Salmonella*, comparable to *Saccharomyces boulardii* strains, emphasizing the need for further exploration of *K lactis*' antibacterial potential due to the limited studies available on this aspect.

Conclusion

The exploration of the compatibility between *K lactis* and probiotic *Lactobacillus* has revealed symbiotic characteristics, suggesting the potential of *K lactis* as a probiotic yeast alongside established probiotic *Lactobacillus* species. The mutual enhancement of their growth was evident at the intersection of their cultures, resulting in denser growth compared to individual cultures. In terms of antimicrobial potential, *K lactis* did not exhibit antibacterial activity. Conversely, *L rhamnosus*, and *L plantarum* demonstrated the highest antibacterial activity among the various *Lactobacillus* species. On the other hand, *L acidophilus*, *L helveticus*, and *L bulgaricus* exhibited relatively lower activity compared to the former strains. This study suggests that *K lactis* can be effectively combined with other probiotic *Lactobacillus* species to produce novel functional products with enhanced benefits.

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